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Carnitine prevents the early mitochondrial damage induced by methylglyoxal bis(guanylhydrazone) in L1210 leukaemia cells

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We previously found that the anti-cancer drug methylglyoxal bis(guanylhydrazone) (mitoguazone) depresses carnitine-dependent oxidation of long-chain fatty acids in cultured mouse leukaemia cells [Nikula, Alhonen-Hongisto, Seppänen & Jänne (1984) *Biochem. Biophys. Res. Commun.* **120**, 9–14]. We have now investigated whether carnitine also influences the development of the well-known mitochondrial damage produced by the drug in L1210 leukaemia cells. Palmitate oxidation was distinctly inhibited in tumour cells exposed to 5 μM-methylglyoxal bis(guanylhydrazone) for only 7h. Electron-microscopic examination of the drug-exposed cells revealed that more than half of the mitochondria were severely damaged. Similar exposure of the leukaemia cells to the drug in the presence of carnitine not only abolished the inhibition of fatty acid oxidation but almost completely prevented the drug-induced mitochondrial damage. The protection provided by carnitine appeared to depend on the intracellular concentration of methylglyoxal bis(guanylhydrazone), since the mitochondria-sparing effect disappeared at higher drug concentrations.

Although methylglyoxal bis(guanylhydrazone) (mitoguazone) is a potent inhibitor of eukaryotic *S*-adenosylmethionine decarboxylase (EC 4.1.1.50) (Williams-Ashman & Schenone, 1972) and hence retards the accumulation of spermidine and spermine, it is by no means proved that the prevention of polyamine accumulation is responsible for the profound antiproliferative action exerted by the compound. In addition to the effects directed towards the metabolism of the polyamines, methylglyoxal bis(guanylhydrazone) also affects the function and integrity of mitochondria. Pine & DiPaolo (1966) reported that the drug behaves as a mitochondrial poison, inhibiting cellular respiration and uncoupling oxidative phosphorylation. Pathak *et al.* (1977) presented the first evidence indicating that an exposure of L1210 cells to methylglyoxal bis(guanylhydrazone) results in profound mitochondrial damage preceding the inhibition of growth. Since then, this finding has been confirmed and extended. In spite of the extensive documentation, the mechanism of the drug's action on mitochondria remains unsolved. Actively proliferating cells appear to be more prone to develop methylglyoxal bis(guanylhydrazone)-induced mitochondrial damage than are quiescent cells (Mikles-Robertson *et al.*, 1979), which, however, may only be an indication of a more effective accumulation of the drug in rapidly proliferating cells (Jänne *et al.*, 1981). The compound appears to inhibit selectively mitochondrial DNA synthesis (Feuerstein *et al.*, 1979), yet a direct cause-and-effect relationship between this inhibition and morphological damage remains to be established. Experimental evidence is also available suggesting that bis(guanylhydrazones) by binding at the mitochondrial inner membrane may interfere with cation (K^+?) binding and produce functional impairment (Byczkowski *et al.*, 1981). Methylglyoxal bis(guanylhydrazone) is likewise reported to compete with the natural polyamines for negatively charged binding sites at the mitochondrial membrane (Byczkowski & Porter, 1983). Moreover, mitochondrial damage in the intestinal epithelium is suggested to be responsible for the antiproliferative toxicity of the drug (Pleshkewych *et al.*, 1983).

In addition to the above-cited effects on mitochondria, we found (Nikula *et al.*, 1984) that methylglyoxal bis(guanylhydrazone) inhibited the mitochondrial oxidation of long-chain fatty acids. Interestingly, this inhibition was competitive in respect to carnitine (Nikula *et al.*, 1984). As carnitine partly reversed the inhibition of palmitate oxidation in cells exposed to the drug (Nikula *et al.*, 1984), we have now investigated whether

carnitine also protects the cells against the drug-induced mitochondrial damage. Under the conditions where carnitine reversed the inhibition of palmitate oxidation produced by methyglyoxal bis(guanylhydrazone), it also strikingly protected mitochondria from early ultrastructural damage. This protective effect, however, was lost when the intracellular concentration of the drug rose to about 1000amol/cell.

Experimental

Cell cultures

Mouse L1210 cells were grown in RPMI1640 medium (Gibco, Paisley, Scotland, U.K.) with 5% (v/v) pooled human serum (Finnish Red Cross Transfusion Service, Helsinki, Finland) and antibiotics (penicillin and streptomycin, 50μg/ml each).

Chemicals

Methylglyoxal bis(guanylhydrazone) dihydrochloride was obtained from Orion Pharmaceutical Co. (Espoo, Finland). [1-^{14}C]Palmitate (sp. radioactivity 56Ci/mol) was purchased from Amersham International (Amersham, Bucks., U.K.). DL-Carnitine and defatted albumin were obtained from Sigma (St. Louis, MO, U.S.A.).

Analytical methods

The oxidation of palmitate, bound to defatted albumin (1:68, w/w), was determined in tumour cell suspension (in phosphate-buffered saline) as described by Nikula *et al.* (1984). The reproducibility of the assay within the same experiment was very good ($\pm$10%), but varied between experiments, depending on the metabolic state of the cells. Intracellular contents of methylglyoxal bis(guanylhydrazone) were determined by the enzyme-inhibition assay of Seppänen *et al.* (1980).

Preparation of cells for electron microscopy

The cell pellets were fixed with 2.5% (v/v) glutaraldehyde in 0.1M-phosphate buffer (pH7.3) for 45min at room temperature. The cells were washed with the phosphate buffer and treated with a solution containing 1% OsO_4 for 90min. Dehydration of the fixed cell pellet was accomplished with graded ethanol treatments and the dehydrated pellet was finally mounted into epoxy resin and sliced for electron microscopy. The sliced sections were stained with lead citrate and uranyl acetate.

Results

As shown in Table 1, exposure of L1210 cells to 5μM-methylglyoxal bis(guanylhydrazone) for 7h depressed palmitate oxidation by about 50%. This inhibition was almost totally prevented by 2mM-DL-carnitine (Table 1). As also indicated in Table 1, carnitine did not have any effect on the intracellular accumulation of methylglyoxal bis(guanylhydrazone). Carnitine also strikingly protected the cells against ultrastructural mitochondrial damage induced by the drug, as more than 50% of the mitochondria were damaged in the absence of carnitine, in comparison with less than 1% damaged mitochondria counted in cells supplemented with carnitine (Table 1). Electron micrographs depicted in Fig. 1 (parts *a* and *b*) were from the experiment presented in Table 1. The mitochondria of tumour cells exposed to methylglyoxal bis(guanylhydrazone) were greatly swollen and had almost totally lost their normal structure (Fig. 1*a*), whereas mitochondria in cells exposed to the drug in the presence of carnitine were apparently morphologically normal (Fig. 1*b*).

In another experiment, the tumour cells were exposed to methylglyoxal bis(guanylhydrazone) in the absence or presence of carnitine for a slightly longer period. The intracellular drug concentrations now exceeded 1000amol/cell (the uptake of

Table 1. *Effect of methylglyoxal bis(guanylhydrazone) in the absence or presence of* DL-*carnitine on the oxidation of palmitate and on mitochondrial morphology in L1210 leukaemia cells*

The cells were grown in the absence or presence of 5μM-methylglyoxal bis(guanylhydrazone) (MGBG) without or with 2mM-DL-carnitine for 7h, after which palmitate oxidation was measured and samples for electron microscopy were prepared.

Treatment	Palmitate oxidation (pmol/10^6 cells)	MGBG concn. (amol/cell)	Damaged mitochondria
None	273	—	—*
DL-Carnitine	327	—	—*
MGBG	141	686	62/120 (52%)
MGBG + DL-carnitine	230	675	1/173 (0.6%)

* No evidence for mitochondrial damage (swollen electrolucent mitochondria with or without distortion of the cristae were considered damaged).

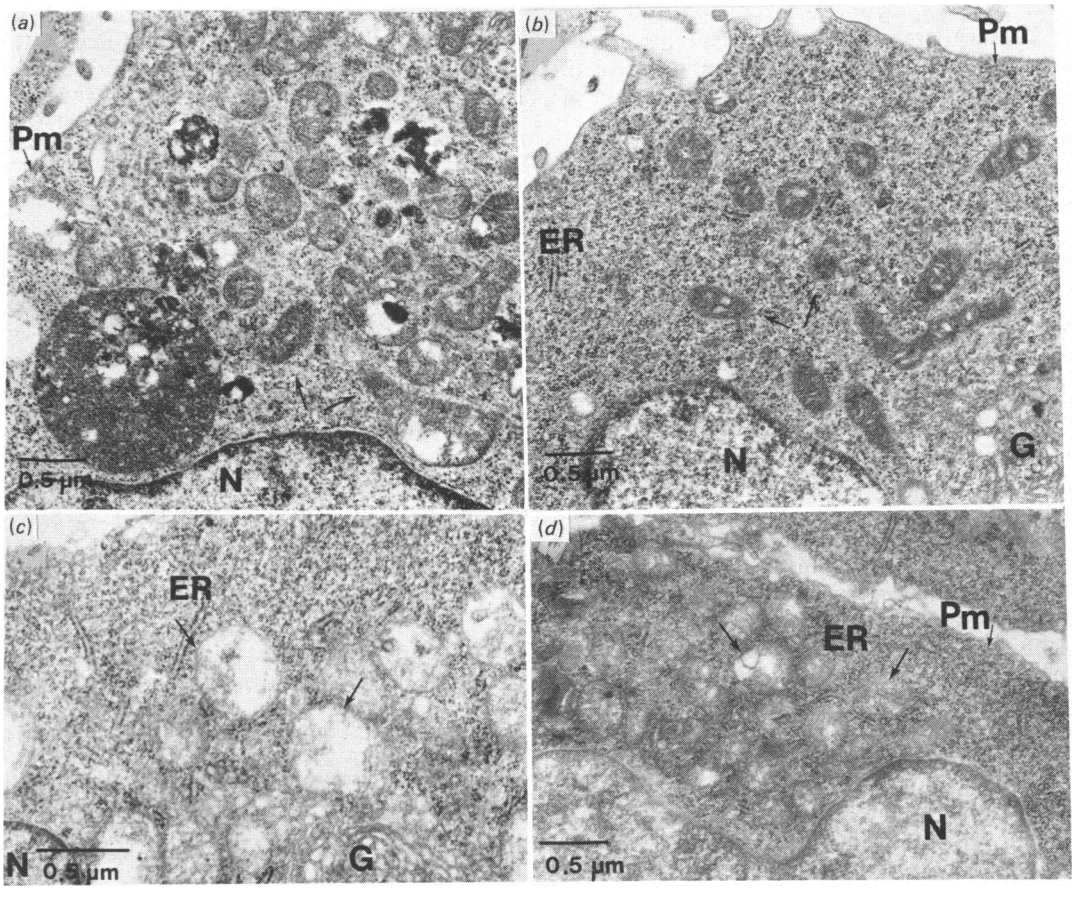

Fig. 1. *Electron micrographs of L1210 cells exposed to 5 μM-methylglyoxal bis(guanylhydrazone) for 7 h (a and b) or for 8 h (c and d) in the absence (a and c) or in the presence (b and d) of 2 mM-DL-carnitine*

The intracellular concentrations of methylglyoxal bis(guanylhydrazone) were (*a*) 686, (*b*) 675, (*c*) 1050, and (*d*) 1060 amol/cell. Key: Pm, plasma membrane; N, nucleus; ER, endoplasmic reticulum; G, Golgi apparatus. Magnification: (*a*), (*b*) and (*d*) ×21600; (*c*), ×30000.

the drug is critically dependent on the growth rate of the cells; even a slight increase in the growth rate results in higher intracellular drug concentration). Practically all (96%) the mitochondria were severely damaged in cells exposed to the drug in the absence of carnitine. Distinct mitochondrial damage (present in 38% of the mitochondria) was also evident in cells exposed to methylglyoxal bis(guanylhydrazone) in the presence of carnitine, although the damage was not nearly as total as without carnitine.

Fig. 1 (parts *c* and *d*) shows the ultrastructural changes in the same tumour cells described above: both the severity and the extent of the mitochondrial damage were more pronounced in cells exposed to methylglyoxal bis(guanylhydrazone) in the absence (Fig. 1*c*) than in the presence of carnitine (Fig. 1*d*).

After longer periods of exposure and at high intracellular drug concentrations, the protective effect of carnitine was totally lost and the inhibition of palmitate oxidation became irreversible.

Discussion

Methylglyoxal bis(guanylhydrazone) is a potent cytotoxic drug that is currently undergoing clinical re-evaluation for its use in the treatment of human cancer (Warrell & Burchenal, 1983; Jänne *et al.*, 1983). Although the exact mode of action is still incompletely understood, it is believed that the cytotoxicity of methylglyoxal bis(guanylhydrazone) is related to its ability to inhibit polyamine biosynthesis (Williams-Ashman & Schenone, 1972) and to the fact that the drug behaves as a mitochondrial poison (Pathak *et al.*, 1977). Although the morphological features of methyl-

glyoxal bis(guanylhydrazone)-induced mitochondrial damage are well documented, practically nothing is known about the biochemical basis of the selective (Pathak *et al.*, 1977) destruction of mitochondria, but not of other cell organelles.

The fact that methylglyoxal bis(guanylhydrazone) inhibits the oxidation of long-chain fatty acids (Nikula *et al.*, 1984) and carnitine not only overcomes this inhibition but also prevents the early mitochondrial damage produced by the drug may indicate that impaired fatty acid oxidation could be one of the mechanisms leading to the mitochondrial damage.

The finding that carnitine protects cells against the toxicity of methylglyoxal bis(guanylhydrazone) may likewise offer practical applications. In fact, preliminary experiments (P. Nikula, H. Ruohola, L. Alhonen-Hongisto & J. Jänne, unpublished work) have shown that carnitine given concomitantly with lethal doses of methylglyoxal bis(guanylhydrazone) significantly protects mice against drug-induced death.

As there appears to be a certain threshold concentration at which the protection by carnitine disappears, the protective effect may be directed to tissues in which the accumulation of the drug is slow, such as parenchymal organs and muscle tissues (Kallio *et al.*, 1983). Especially interesting is the view that the frequently reported muscle symptoms during the treatment of human patients with methylglyoxal bis(guanylhydrazone) (Warrell & Burchenal, 1983; Jänne *et al.*, 1983) are based on an impaired fatty acid oxidation and could thus be alleviated with the administration of carnitine, which, in fact, is registered in many countries for human use.

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